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Methicillin-resistant *Staphylococcus aureus* CC398 isolates with indistinguishable *ApaI* restriction patterns in colonized and infected pigs and humans

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Sir,
Pigs have recently been demonstrated as a source of the methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398, responsible for human infections in several countries.^{1,2} Recent evidence of an invasive infection caused by a multiresistant MRSA ST398 strain of swine origin in a pig-farm worker reinforces the emerging problem of animal MRSA as a human occupational health risk.³

In May 2009, an outbreak of exudative epidermitis (EE) in three pig farms in Portugal affected ~15% of the 6-week-old weaned pigs and resulted in an increased mortality rate (20%). Two farms were farrow-to-finish sites with ~200 sows (Farms A and B); the third one was a farrow-to-weaners site with ~250 sows (Farm C). Infected pigs had been unsuccessfully treated with cefotiofur and amoxicillin. In Farm C, three pigs were additionally treated with enrofloxacin. The diagnosis of EE was based on clinical signs. Skin lesion swabs were sent to the Faculty of Veterinary Medicine, Technical University of Lisbon (2, 3 and 11 swabs from Farms A, B and C, respectively). These swabs were directly streaked on Columbia 5% blood sheep agar and MacConkey agar plates (bioMérieux, Marcy l'Étoile, France). No growth was seen on MacConkey agar, but large numbers (ranging from 300 to >1000 colonies per plate) of haemolytic white colonies were found on the sheep blood

agar, indicating a high skin bacterial load. These colonies were confirmed as *S. aureus* by Gram staining, catalase and coagulase testing, and the BBLTM CrystalTM Gram-positive typing system (BD Diagnostic System, USA). *Staphylococcus hyicus*, which normally causes acute skin infection in suckling and weanling pigs, was not found. MRSA was identified by plating on a selective medium, Chrom MRSA ID (bioMérieux), and by polymerase chain reaction (PCR) for the *mecA* and *nuc* genes (<http://www.crl-ar.eu>).

Additionally, at each farm, MRSA colonization was screened in healthy pigs. A pool of five nasal swabs was randomly collected from pigs of each of the following age groups: sows in the gestation unit; sows in the farrowing unit; suckling pigs; and weanling pigs. Nasal swabs were also obtained from the attending veterinarian of the three farms and farm workers ($n=10$), on a voluntary basis. Swabs underwent selective enrichment and MRSA isolates were identified by PCR for the *mecA* and *nuc* genes (<http://www.crl-ar.eu>). All MRSA isolates from infection and colonization were subjected to two specific PCRs for the detection of *S. aureus* ST398,⁴ *spa* typing (<http://spaserver.ridom.de>) and SCCmec typing, as previously performed.⁵ Isolates were also tested for the *lukF/lukS* genes.² Clonality was assessed by PFGE with *ApaI*⁶ and *Cfr9I*⁴ restriction. MICs of 18 antimicrobials were determined by broth microdilution (MicroScan PM21; Dade Behring, Deerfield, IL, USA) (Table 1), and interpreted according to CLSI guidelines M31-A3 and M100-S20. MICs of oxacillin were >4 mg/L and of penicillin were >8 mg/L for all MRSA strains.

Table 1 presents the characterization of the MRSA isolates found in this study. Thirteen out of 16 skin lesions swabs had a high MRSA bacterial load and 3 were negative. These samples were recovered from the three pigs under enrofloxacin treatment. Out of the 10 farm workers tested, 4 were carriers, as well as the attending veterinarian. Among the healthy pigs, only 2 out of 12 pooled samples were positive and those were found in the sows groups (Farm A, gestation and farrowing unit). The low prevalence of MRSA colonization among the healthy pigs might be explained by the low sensitivity associated with the sampling pooling method. Three different *spa* types were isolated: two dominant (t011 and t4571, tandem repeat sequences 08-16-02-25-34-24-25 and 08-34-24-25, respectively); and one only found in the veterinarian (t1255, 08-16-34-24-25). These *spa* types are related, since t1255 and t4571 differ by two and three tandem repeat deletions from *spa* type t011, respectively. Furthermore, all MRSA isolates were identified as ST398 by specific PCR, had indistinguishable PFGE *ApaI* patterns (not all strains were typeable by *Cfr9I*) and harboured SCCmec type V. PFGE results demonstrated clonality among the MRSA strains isolated from healthy and infected pigs, farm workers and the attending veterinarian. Therefore, MRSA transmission is likely to have occurred between the different hosts, animals and humans. All three farms in this study

Table 1. Characterization of MRSA isolates from pigs with exudative epidermitis, sows, attending veterinarian and farm workers from three farms sampled in Portugal

Farm	Sample source	Sample site	Resistance pattern ^d	<i>spa</i> type	PFGE type (ApaI restriction)
A	pig EE ^a	skin lesion	CLI, ERY, OXA, PEN, TET	t011	A
	pig EE ^a	skin lesion	CLI, ERY, OXA, PEN, TET	t011	A
	sows—gestation ^b	nasal swab	CLI, ERY, OXA, PEN, TET, SXT	t011	A
	sows—farrowing ^b	nasal swab	CLI, OXA, PEN, TET	t011	A
	farm worker ^c	nasal swab	CLI, ERY, OXA, PEN, TET	t011	A
	farm worker ^c	nasal swab	CLI, ERY, OXA, PEN, TET	t011	A
	farm worker ^c	nasal swab	CLI, ERY, OXA, PEN, TET, SXT	t011	A
B	pig EE ^a	skin lesion	CLI, OXA, PEN, TET	t011	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET	t011	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET	t011	A
	farm worker ^c	nasal swab	OXA, PEN, TET	t011	A
C	pig EE ^a	skin lesion	CLI, OXA, PEN, TET, SXT	t011	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET, SXT	t011	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET	t4571	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET, SXT	t011	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET, SXT	t4571	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET, SXT	t011	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET	t011	A
	pig EE ^a	skin lesion	CLI, OXA, PEN, TET	t011	A
A, B, C	attending veterinarian	nasal swab	OXA, PEN, TET	t1255	A

CLI, clindamycin; ERY, erythromycin; OXA, oxacillin; PEN, penicillin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole.

^aIndividual sample collected from a weanling pig showing clinical signs of exudative epidermitis (EE).

^bPooled sample of five individual nasal swabs.

^cFrom Farms A and C, 3 farm workers were sampled; from Farm B, 4 workers were sampled.

^dAntimicrobial agents were used in the following test ranges: chloramphenicol, 4–16 mg/L; ciprofloxacin, 0.5–2 mg/L; clindamycin, 0.25–2 mg/L; erythromycin, 0.25–4 mg/L; gatifloxacin, 1–4 mg/L; gentamicin, 2–8 mg/L; levofloxacin, 0.5 and 2–4 mg/L; linezolid, 1–4 mg/L; moxifloxacin, 0.12 and 0.5–2 mg/L; netilmicin, 4–16 mg/L; oxacillin, 0.25–4 mg/L; penicillin, 0.03–0.25 and 2–8 mg/L; rifampicin, 0.5–2 mg/L; quinupristin/dalfopristin, 0.5–2 mg/L; teicoplanin, 1–16 mg/L; tetracycline, 2–8 mg/L; trimethoprim/sulfamethoxazole, 2/38 mg/L; and vancomycin, 1–16 mg/L.

received breeders from the same supply farm, which suggests that this might have been the potential source of MRSA CC398. Pantón–Valentine leucocidin genes (PVL) were not detected in this study. Even so, it has been demonstrated that livestock-associated MRSA ST398 PVL-negative strains can still be virulent, and cause human, swine and bovine infections.^{3,6,7} All MRSA strains ($n=20$) were resistant to tetracycline, as commonly reported in the literature (Table 1).^{2,5,8} Seven isolates were resistant to trimethoprim/sulfamethoxazole (35%), 6 (30%) were resistant to both erythromycin and clindamycin, and 12 (60%) were resistant to clindamycin but not erythromycin. These findings are in agreement with previously reported data on environmental MRSA pig farm isolates in Portugal.⁹ To the best of our knowledge, only one case has been reported before of an outbreak of EE associated with MRSA CC398 in pigs, but the isolation of MRSA from skin infection in pigs has been described.^{6,8} In the present study, all three farms performed systematic use by single intramuscular injection of ceftiofur in 1-day-old pigs (20 mg/piglet, NaxcelTM), and administered antimicrobials in feed among suckling and weanling pigs (colistin sulphate: 3–5 kg/ton; zinc sulphate: 3 kg/ton). The lack of prudent antimicrobial usage in these farms may increase the risk of MRSA colonization or infection. The

emergence of associated co-resistance in clonal MRSA CC398 isolates requires further research.

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Transparency declarations

None to declare.

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High frequency of β -lactam susceptibility in CTX-M-type extended-spectrum- β -lactamase-producing *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis* according to the new CLSI recommendations

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Sir,

Class A extended-spectrum β -lactamases (ESBLs) are a major cause of β -lactam resistance in Gram-negative bacteria.¹ From 2005 to 2009, the CLSI recommended the use of screening and confirmatory phenotypic tests for ESBL detection in *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Proteus mirabilis* with reduced susceptibility to some cephalosporins and aztreonam.² Once confirmed as an ESBL producer, the CLSI recommended that all penicillins, cephalosporins and aztreonam should be reported as resistant, regardless of the halo diameter in the disc diffusion test or the MIC.² The rationale for this recommendation was based on a number of studies indicating that infections caused by ESBL-producing strains presented high rates of therapeutic failures when treated with these drugs, despite their *in vitro* susceptibility to some of these agents.^{3–5}

In the 2010 document, the CLSI changed the susceptibility breakpoints (see Table 1 footnote) of some important cephalosporins and aztreonam, and established that if these new criteria are used, then ESBL detection is no longer necessary.³ This new recommendation was based on the inference that these antimicrobials, according to their pharmacokinetic/pharmacodynamic (PK/PD) properties, could be effective against 'low MIC' organisms, regardless of ESBL production.

In this study, we assessed the implications of these new recommendations on the susceptibility reports of a sample of CTX-M-type ESBL-producing organisms.

We assessed a total of 60 isolates of *K. pneumoniae* (32 isolates), *E. coli* (16 isolates) and *P. mirabilis* (12 isolates) that were recovered from patients admitted to the Hospital de Clínicas de Porto Alegre, a Brazilian tertiary-care teaching hospital, from September 2005 to August 2006, which presented screening and confirmatory phenotypic tests positive for ESBL.² The isolates were identified by conventional biochemical tests.⁶ All isolates also contained the CTX-M gene detected by PCR performed as previously described.⁷ The results of the susceptibility profile of these isolates to ceftazidime, ceftriaxone, cefepime and aztreonam, as determined by the disc diffusion method, were evaluated according to the new breakpoints proposed in the 2010 CLSI document.³ Isolates classified as intermediate or susceptible by the disc diffusion method were subjected to MIC determination by Etest[®] (Solna, Sweden).

All isolates proved to be resistant to ceftriaxone. A total of 19 (31.6%), 33 (55%) and 21 (35.0%) CTX-M-type ESBL-producing isolates were either intermediate or susceptible to aztreonam, ceftazidime and cefepime, respectively (Table 1). Thirty-five (58.3%) of the 60 isolates were either intermediate or susceptible to at least one of the antibiotics; of these, 25 (41.7%) would be considered fully susceptible to at least one antibiotic, and, within this group, 8 (13.3%) isolates would be fully susceptible to aztreonam, ceftazidime and cefepime (Table 1). The MIC values of the susceptible isolates ranged from 0.25 to 4.0 mg/L for aztreonam, 0.094 to 4.0 mg/L for ceftazidime and 2.0 to 8.0 mg/L for cefepime.

Our study showed a high rate of cephalosporin and aztreonam susceptibility in CTX-M-type-producing *E. coli*, *K. pneumoniae* and *P. mirabilis* using the new interpretative criteria recommended by the 2010 CLSI document. Additionally, using only the disc diffusion breakpoint without the interpretative